Lower respiratory tract infection caused by respiratory syncytial virus. The short-term and the long-term efficacy of corticosteroids
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Introduction and aims of the study
**History**

More than 45 years ago Morris and colleagues described the recovery of a virus that caused an outbreak of colds with coryza in a colony of chimpanzees. They could not know that their publication would be the beginning of an enormous body of research on the causative virus, that was named chimpanzee coryza agent, by investigators from all over the world. Further research revealed that it was probably from human origin. Based on its cytopathic effects, forming syncytia in tissue culture, the virus was renamed respiratory syncytial virus (RSV). RSV is now recognised to be the most common cause of respiratory infection in infants and young children.

**Virus**

RSV is a non-segmented negative strand RNA virus. It is a member of the family of Paramyxoviridae and classified in the genus of Pneumovirus. It varies in size from 150-300 nm and its genome codes for ten proteins. The G (attachment), the F (fusion) and the SH protein are transmembrane surface proteins that form the major antigenic determinants of the virus.

Based on variation of the G protein and to a lesser extent the F protein subtypes A and B are recognised. Both subtypes may be isolated simultaneously during one winter season but relative frequencies are not constant. Reports on disease severity in relation to subtype of RSV are not consistent. Some reports suggest that subtype A causes more severe disease, whereas others could not confirm this, or found that subtype B causes more severe disease.

**Epidemiology**

RSV is the most common cause of respiratory tract infections in infants and young children throughout the world. RSV infections may occur throughout the year but in countries with a temperate climate there is an annual peak in the winter (Figure). Approximately 70% of all infants are infected with RSV in their first year of life, and by the age of 2 almost all children have experienced at least one infection with RSV. RSV infections occur most frequently in patients between 2 and 7 months of age.

Several patients groups have been recognised as risk groups for a severe course of RSV infection under which premature infants, patients with underlying pulmonary disease such as chronic lung disease or cystic fibrosis, but also patients with congenital heart disease, especially those with pulmonary hypertension. In addition, infants before the post-conceptional age of 44 weeks – either premature born or not – are at increased risk for severe RSV infection.

About 1% to 2% of the RSV infected children needs to be hospitalised for observation and supportive therapy, although this varies with the investigated population.
the Netherlands, the last decade between 800 and 3000 children have been hospitalised annually for bronchiolitis. In about 5% to 8% of the hospitalised children the RSV lower respiratory tract infection (RSV-LRTI) leads to severe respiratory insufficiency necessitating mechanical ventilation.27

Immunity and immunopathology
RSV is not very immunogenic, reinfections occur throughout life. However, reinfections tend to be milder and are limited to the upper airways, indicating that immunity mainly protects the lungs.20,21,28

Humoral immunologic response
After primary infection with RSV mucosal and systemic antibodies are produced.29-31 The levels of systemic IgG, IgM, IgA and IgE are dependent of age, the presence of maternal antibodies and viral epitopes.32 It has been suggested that the humoral immune response may lead to increased pulmonary pathology.33 Indeed, experimental studies have shown that antibodies to the F protein may enhance in vitro infection of human macrophages.34 In addition, Welliver et al demonstrated that RSV-specific IgE is associated with wheezing during lower respiratory tract infection and that high titres of IgE correlated with bronchial reactivity in later life.35,36 On the other hand, these observations are not supported by several epidemiological and experimental animal studies, as well as clinical prevention trials with immunoglobulins that have given clear evidence that the risk of severe RSV infection is inversely related to the level of neutralising serum immune globulins.16,28,37-42 In particular, serum antibodies against the F and G protein play a key role in protection against RSV infections.6,40,43

Cellular immunologic response
While the humoral immune response seems to play a role in the protection against RSV infection, the cellular immune response is thought to be of major importance in the clearance of the virus. Both experimental and human studies have demonstrated that immune competent hosts are able to clear the virus after natural infection within 3 weeks, whereas immune compromised hosts with cellular immunodeficiencies tend to have more severe disease as well as prolonged viral shedding.44-47 The attraction and activation of inflammatory cells to the respiratory tract is in great extent orchestrated by the respiratory epithelial cells. Experimental studies have demonstrated that upon exposure to RSV respiratory epithelial cells are able to release several pro- and anti-inflammatory cytokines as well as chemotactic mediators such as interleukin(IL)-1β, IL-1α, IL-6, IL-8, RANTES, macrophage inflammatory protein-1α (MIP-1α), monocyte chemotactic protein (MCP-1) and tumor necrosis factor(TNF)-α.48-53
In addition, in vivo studies have demonstrated that several of these cytokines and chemokines such as, IL-8, RANTES, MIP-1α and MCP-1 are present in upper and lower respiratory tract during RSV-LRTI.51,52,54-56 Once released these cytokines induce the migration and activation of several inflammatory cells. IL-8 is an important chemoattractant for neutrophils.57 RANTES, MIP-1α and MCP-1 are chemotactic and activation factors for eosinophils,58 monocytes,53 but also for several T-cell subpopulations.59,60 The role of both neutrophils and eosinophils during RSV disease is supported by their autocrine regulated release of IL-8 and RANTES upon exposure to RSV.53,61 Clinical studies on cell morphology have demonstrated that neutrophils are far out the most predominant cells in the airways during RSV-LRTI.54,62,63 Their contribution to the immune response is further supported by the finding of their enzymatic active mediator elastase in the airways during RSV disease.56 Although eosinophils are found in very low numbers in the airways, they very likely participate also in the immune response against RSV, since eosinophil cationic protein (ECP) is demonstrated in both the upper and lower airways, as well as in serum during RSV-LRTI.29,52,64-67 Moreover, in vitro studies have shown that RSV is able to activate and prime eosinophils to release several mediators such as superoxide, leukotriene C4 and ECP.68,69 The discrepancy between the low number of eosinophils in the airways and the abundant presence of their active proteins may be explained by the tissue dwelling character of eosinophils.70,71 Much about T-cell responses in RSV infection has been learned from rodent studies, whereas less is known about the T-cell patterns in humans during RSV infection. From the animal studies it has become clear that viral infection of the lungs causes an influx of T-lymphocytes, that are initially of the CD4 phenotype, whereas CD8 cells predominate at the time of virus elimination.65 Controversy exists about the specific role of the T-cell subsets. Some authors found that CD8 cells were more antiviral but at the same time led to more immunopathogenesis compared to CD4 cells,72 whereas other found the contrary.73 T-helper cells are able to produce exclusive patterns of cytokines and based on these patterns can be divided in at least 2 subsets. Th-1 cells are characterised by the production of interferon-γ and IL-2, whereas Th-2 cells produce IL-4, IL-5, IL-10 and IL-13 giving rise to IgE production and eosinophil recruitment and activation. In most infectious diseases Th-1 cytokine production is associated with protection, whereas Th-2 cytokine patterns lead to disease progression.74 In animal models it has been shown that natural infection with RSV leads to a Th-1 response.75,76
Immunopathology

Shortly after its discovery as an important respiratory pathogen it has become clear that pathogenesis of RSV-LRTI is not only the result a direct cytotoxic effect of RSV, but that the immune response against RSV contributes to the pathogenesis. The first indication for this role of the immune response came from the experiences with formalin-inactivated virus (FI-RSV) vaccine that was used in the 1960s. After vaccination with FI-RSV children developed severe lower respiratory tract infection when subsequently infected with RSV the next season. In the lungs of two lethal cases peribronchiolar infiltrates of mononuclear cells with some excess in eosinophils was found. The mechanism responsible for these vaccine-associated enhanced disease is still not completely understood. Neutralising activity of the antibodies against F protein proved to be low in FI-RSV vaccinated children compared to naturally infected controls. It has been suggested that formalin inactivation of the virus changes epitopes within the F and G protein that normally generate the neutralising antibodies. However, Murphy et al demonstrated that in cotton rats antibodies generated by purified F and G protein vaccines had similar low neutralising activity when compared to FI-RSV vaccinated animals, but did not induce potentiation of pulmonary pathology, suggesting that low neutralising activity alone seems to be insufficient to cause enhancement of pulmonary histopathology.

The type of T-cell response may also play a role. It has been shown that natural RSV infection stimulates T helper cells with a Th-1 cytokine profile resulting in CD8 cytotoxic T-cells, whereas inactivated virus or G protein elaborates a Th-2 response that in turn may lead to a more exaggerated eosinophil influx and inflammation in the lung. In addition, from animal studies it became clear that priming with wild type RSV elaborates a Th-1-like response upon reinfection whereas priming with formalin inactivated virus leads to a Th-2-like response upon reinfection, giving rise to increased airway inflammation.

A second indication for the immune mediated character of RSV-LRTI can be found in the similarities between RSV bronchiolitis and asthma, a typical immune mediated disease. Asthma and RSV bronchiolitis have both clinical and histopathological features in common. In addition, the inflammatory response provoked in respiratory epithelial cells by RSV on several aspects closely resembles that of the response that occurs after exposure to allergens.

Finally, experimental studies have shown that also during natural infection the immune response may contribute to enhanced disease. In an experimental model Cannon et al infected immune deficient mice with RSV. They demonstrated that only those mice that received RSV-specific cytotoxic T-cells concomitant with the virus died. Graham et al demonstrated less morbidity in CD4 and CD8 T-cell deficient mice after they were infected with RSV when compared with immune competent mice.
Symptomatology
The infection usually starts with coryza and cough and low grade fever. In about 30% of the cases also the lower airways become involved after a few days and bronchiolitis or pneumonia may develop. RSV-LRTI potentially causes severe respiratory insufficiency with tachypnea, dyspnea and cyanosis. Hyperinflation, atelectasis and wheezing are the hallmarks of bronchiolitis, whereas pneumonia is characterised by interstitial infiltration, alveolar filling and consolidation. However, it may be very difficult to discriminate between these 2 entities on clinical grounds. RSV infection in newborn infants is rare and may present with apnea. RSV-LRTI may be confirmed with an immunofluorescence or enzyme-linked immunosorbent antigen assay of nasopharyngeal aspirate. Children recover in a few days to 1 week from infection. In industrialised countries the mortality of children hospitalised for RSV-LRTI has been reported between 1% to 3%, although this varies greatly with the investigated population.

Long-term airway morbidity
RSV-LRTI is strongly associated with increased airway responsiveness and wheezing during the ensuing years (post-bronchiolitic wheezing). Some authors report even the development of asthma later in childhood. The pathogenesis of this long-term morbidity is still not clear. Both genetic predisposition as direct viral and immunological injury during the acute infection have been suggested as possible explanations.

Treatment and prevention
Therapy of RSV-LRTI is mainly supportive since no effective treatment is available. Antiviral, bronchodilator and anti-inflammatory therapy for RSV-LRTI have been subject of a large number of clinical trials. These trials have shown conflicting results, which contributes to an inconsistent approach of patients with RSV infection world-wide.

Much effort has been undertaken in the development of a vaccine against RSV, but until now no effective vaccine is available. Immunoprophylaxis has been proven to be an effective alternative in the prevention of RSV disease in patients that belong to certain risk groups for severe RSV disease.
Aims of the study
The immune mediated character of RSV disease supports the potential role of immune modulating drugs like corticosteroids in the treatment of RSV-LRTI. Immunopathologic phenomena not only play a role during the acute infection but potentially also contribute to development of long-term airway morbidity after the acute infection. The principal aim of the thesis is to further assess the short-term and long-term effects of corticosteroids in RSV-LRTI.

Outline of the thesis
In general, less than 2% of the children with RSV disease needs to be hospitalised. However, the number of bronchiolitis admission in the Netherlands has increased in the 1980s to approximately 1000 admissions annually. To assess if this trend continued in the decade thereafter an analysis of the annual bronchiolitis hospitalisations from 1991 through 1999 was performed. The results of this analysis are subject of Chapter 1.

No effective treatment is available for RSV disease. Although several treatment strategies have been investigated in a large number of trials, these trials have only shown conflicting results. In Chapter 2 we present a review of these trials. The results of a randomised controlled trial in which the efficacy of corticosteroids in the treatment of patients hospitalised with RSV-LRTI was evaluated are presented in Chapter 3. In this trial we found that corticosteroids are effective in patients with severe RSV-LRTI, such as those patients that need mechanical ventilation. These results formed the base of a new trial in which the effect of corticosteroids in patients mechanically ventilated for RSV-LRTI was evaluated. The results of that randomised controlled trial are presented in Chapter 4. In an attempt to understand the clinical effects of corticosteroids in RSV-LRTI from a immunological point of view an analysis of the effect of corticosteroids on airway inflammation was made in a subcohort of patients that were included in that clinical trial. The results of that analysis are discussed in Chapter 5.

The effects of corticosteroids on long-term airway morbidity after RSV-LRTI were evaluated in a follow up study, of which the results are presented in Chapter 6.

Finally we give a contemplation on the lack to control RSV infection in Chapter 7.
Number of positive RSV isolations from 17 laboratories in the Netherlands (data obtained from Dutch Working Group on Clinical Virology in co-operation with the National Institute of Public Health and the Environment)

References
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